

Exhibit B

NOTEBOOK NO. 2233
ISSUED TO R. Saito
ON _____
DEPARTMENT m3
RETURNED 19
19

— SCIENTIFIC NOTEBOOK CO. —
5007 WEST DONNA DRIVE
STEVENVILLE, MICHIGAN 49127

Obtained ~100 μ l of second prep Tag polymerase from David. (heparin-Sepharose, frac 52-53, concentrated.)

20 mM Tris-Cl pH 8

0.1 mM KCl

0.1 mM EDTA

1 mM DTT

50% Glycerol

7400-11,700 μ /ml

mM Salmon Sperm DNA.

}

Storage buffer
(note: no gelatin)

Variation in concentration reflects results obtained in two ~~of~~
assays using different dNTP stocks. Higher value (12 μ / μ l)
with assay components used in assay of ~~first~~ first batch
Tag polymerase (2167:65).

If variation in μ / μ l depending on DNA substrate is same as before, concentration with M13 primer extension is 21 μ / μ l!

This stuff is ~3x more concentrated than first lot.

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(lot 2)

Assay Tag polymerase₁ for activity in PCR amplification of β -globin viaid using Molt4 and PC03/04. Vary # cycles (15-30) and amt. enzyme used (1-4 μ l). Also include lot 1 polymerase as control.

15 20 25 30 ~ #cycles

A B C D:	4 μ l	lot 1	}
E F G H:	1	lot 2	
I J K L:	2	"	
M N O P:	4	"	

25 μ l per sample
containing 250 ng genomic DNA

Molt4 @ 100 μ g/ml, PC03 and PC04 @ 10 μ M, dNTP @ 40 mM

50 μ l DNA (5 μ g)

50 μ l 10x salts

Split into four 100 μ l samples.

50 μ l 2 mg/ml gelatin

Heat \rightarrow 20', 98°

50 μ l PC03

Cool to room temp and add amounts of enzyme indicated above each

50 μ l PC04

Sub-divide further into four 25 μ l samples and subject to indicated number of cycles. (After overlaying with mineral oil.)

50 μ l DMSO "

75 μ l dNTP

125 μ l H₂O

500 μ l

program: 2 1/2' @ 98°

5' @ 37°

A, E, I, M \rightarrow 14 cycles

B, F, J, N \rightarrow 19 "

C, G, K, O \rightarrow 24 "

D, H, L, P \rightarrow 29 "

After last cycle, heat 10-15' @ 60° to finish last ~~extend~~ extension.

Extract mineral oil with CHCl₃ and store at -20°

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ck samples of Tag polymerase assay on gel and
if interesting.

3's described page III. Load 5 μ l each sample onto
NuSieve / 0.5% agarose / 1x TBE \rightarrow ON, 15V (~15hr)

ABCDEF GH IJKL MNOP



15 cycles
20 25 30

A B C D	4 μ l	lot 1
E F G H	1	lot 2
I J K L	2	"
M N O P	4	"

Don't see anything with lot 1 polymerase.
Lots of bands with the new stuff.
Amount of between-band streaking
seems to increase with amt. of
enzyme added. No significant
increase in overall fluorescence
25 vs. 30 cycles even with 4 μ l
lot 2 polymerase. May need to
dilute out (<1 μ l) to reduce
complexity and increase specificity.

This is worth blotting.

rinse: ~200ml 0.5N NaOH, 1.5M NaCl \rightarrow 45', RT

without neutralizing, transfer to Genatrane with 20x SSPE
 \rightarrow ON (~16hr)

rinse filter in 20x SSPE and bake \rightarrow 60' @ 80° in vacuum oven.
what filter Z233:112

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Prepare dot blot containing containing PCR samples from Tag polymerase assay described page 111.

Apply 5 μ l each sample to filter: 5 μ l PCR sample
 195 μ l 0.4N NaOH, 25mM EDTA
 200 μ l

Rinse each well with 0.4 ml 20xSSPE, then entire filter in 20x.

Bake 1 hr @ 80°, in vacuum oven.

Label filter 2233:113

RS18 @ 0.057 pmol/ μ l, 0.5 pmol \rightarrow 8.8 μ l (page 107)

Prehyb: 10ml 5xSSPE, 5xDET, 0.5% SDS \rightarrow 15', 55°

Hyb: above + 0.5 pmol RS18 \rightarrow 60', 55°

Wash: 3x 100 ml 2xSSPE, 0.1% SDS \rightarrow 5-10', RT each

1x 100 ml 5xSSPE, 0.1% SDS \rightarrow 5', 60°

15	20	25	30	exp/loc
•	•	•	•	4 μ l 6x1
•	•	•	•	1 μ l 6x2
•	•	•	•	2 μ l -
•	•	•	•	4 μ l -

5	20	25	30	exp/loc
•	•	•	•	4 μ l 6x1
•	•	•	•	1 μ l 6x2
•	•	•	•	2 μ l -
•	•	•	•	4 μ l -

16 June 86
 Filter: 2233:113
 probe: RS18
 exp: O/H, one screen (-1hr)
 ref: 2233:113

16 June 86
 Filter: 2233:113
 probe: RS18
 exp: 60', one screen
 ref: 2233:113

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Repeat Tag pol assay essentially as described page III.
except ~~use~~ with amounts of enzyme $\leq 1\mu\text{l}$.

A B C D : $1\mu\text{l}$ (lot 2)

E F G H : $1/2\mu\text{l}$ "

A, E, I, M, Q, U : 15 cycles

I J K L : $1/4\mu\text{l}$ "

B, F, J, N, R, V : 20 "

M N O P : $1/8\mu\text{l}$ "

C, G, K, O, S, W : 25 "

Q R S T : $1/16\mu\text{l}$ "

D, H, L, P, T, X : 30 "

U V W X : $1/32\mu\text{l}$ "

Molt4 @ $100\mu\text{g}/\text{ml}$, PCO3 and PCO4 @ $5\mu\text{M}$, dNTP @ $40\mu\text{M}$, gelatin @

$80\mu\text{l}$ DNA

$80\mu\text{l}$ 10X salts Divide into five $100\mu\text{l}$ and one $200\mu\text{l}$ samples and heat $\rightarrow 10'$, 98°

$80\mu\text{l}$ gelatin Cool to RT and add to $200\mu\text{l}$ sample

$80\mu\text{l}$ PCO4 add $2\mu\text{l}$ Tag pol (lot 2).

$80\mu\text{l}$ DMSO Prepare five 2-fold $100\mu\text{l}$ serial dilutions in remaining 5 tubes.

$105\mu\text{l}$ dNTP (Add $5\mu\text{l}$ DNA / $100\mu\text{l}$ (range: $1\mu\text{l}$ to $1/16\mu\text{l}$ Tag pol per tube))

$200\mu\text{l}$ H₂O Sub-divide each further into four $25\mu\text{l}$ samples and overlay with mineral oil (total: 24 tubes)

Subject to 14-29 cycles : $2\frac{1}{2}' @ 98^\circ$ \leftarrow new program
 $3' @ 37^\circ$ $3' @ 37^\circ$ instead of 5 cycles

After last cycle incubate additional $10' @ 60^\circ$ to finish off final extension.

Extract mineral oil with CHCl_3 . Store at -20°

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Hybridize Southern of Tag polymerase assay with RS18.

RS18 @ 0.057 pmol/ μ l, 0.5 pmol \rightarrow 8.8 μ l (page 107)

Prehyb: 20ml 5xSSPE, 5xDET, 0.5% SDS \rightarrow 45', 55°

Hyb: above + 0.5 pmol RS18 \rightarrow 90', 55°

Wash: 3x 100ml 2x SSPE, 0.1% SDS \rightarrow ~5', RT each

1x 100ml 5x SSPE, 0.1% SDS \rightarrow ~15', ~~55~~ 60°

Hyb: 2233:113, 117 with RS18

Project No. _____

TITLE Dot Blot: Tag Pol Assay (Lot 2) - II

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Prepare dot blot containing PCR samples from Tag polymerase assay with $\leq 1\mu\text{l}$ enzyme per reaction.

Samples described page 115.

Apply 5 μl each sample to filter: 5 μl PCR sample
195 μl 0.4N NaOH, 25mM
200 μl

Work-up as described page 113.

Label filter 2233:117

Hyb 1A

Hybridized RS18 to filter above after adding with 1 μl of
2R3A NB

Hybridize RS18 to filter above plus 2233:113 as positive control.

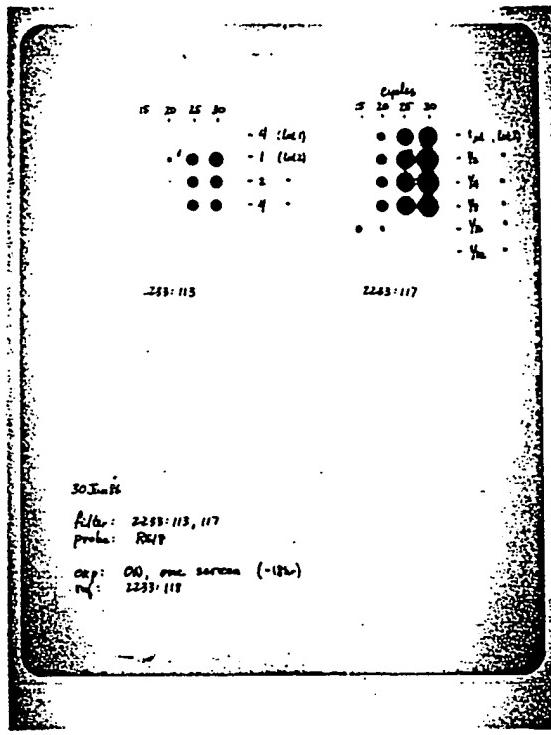
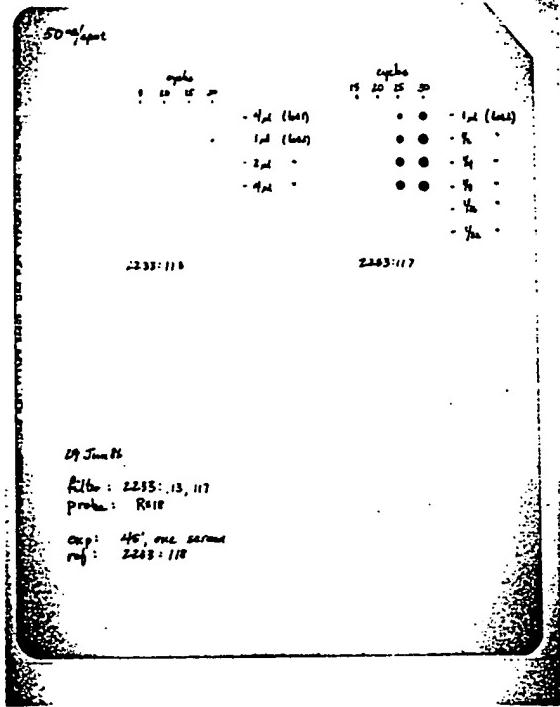
RS18 @ 0.057 pmol/ μl , 0.5 pmol \rightarrow 8.8 μl (p.107)

Prehyb: 10ml 5xSSPE, 5xDET, 0.5% SDS \rightarrow 15' ^{15'} ~~bed~~, 55° (both filter)

Hyb: above + 0.5 pmol RS18 \rightarrow 3hr, 55° in same be

Wash: ~~IRX~~ 3x 100ml 2xSSPE, 0.1% SDS \rightarrow ~5', RT each

1x 100ml 5xSSPE, 0.1% SDS \rightarrow 20', 60°



¹¹ [✓] (with lot 1)
 $\frac{1}{8}$ μ l is optimal amount of enzyme. As before, there is an abrupt cut-off below optimal \rightarrow no signal with $\frac{1}{16}$ μ l. Signal improves from as amount added per PCR reaction is reduced from 4 μ l to $\frac{1}{8}$ μ l.

Lower intensity of signals on 2233:113 relative to 2233:117 probably due to loss of DNA when former was stripped prior to re-hyb.

Not sure if signals in $\frac{1}{16}$ μ l samples after ON exposure are real. Possible that order of those spots was inverted somehow. But could also be artifact. Whatever it is, it is pretty insignificant when compared to $\frac{1}{8}$ μ l spots.

Clearly, the new PCR program (3' @ 37° vs 5' @ 37°) works just fine.

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